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(54) Title: NEW USE

(57) Abstract: The use of Sema7 A polypeptides and polynucleotides in the design of protocols for the treatment of autoimmune disorders, chronic obstructive pulmonary disease, inflammation, psoriasis, wound healing, tissue repair, irritable bowel syndrome, stroke, atherosclerosis, cancer among others, and diagnostic assays for such conditions. Also disclosed are methods for producing such polypeptides by recombinant techniques.

New Use

Field of the Invention

This invention relates to new uses for polynucleotides and polypeptides encoded by them, to their use in therapy and in identifying compounds which may be agonists, antagonists and /or inhibitors which are potentially useful in therapy, and to production of such polypeptides and polynucleotides.

Summary of the Invention

In one aspect, the invention relates to new uses of Sema7A polynucleotides and polypeptides disclosed in WO99/38885 (SmithKline Beecham). Such uses include the treatment of autoimmune disorders, chronic obstructive pulmonary disease, inflammation, psoriasis, wound healing, tissue repair, irritable bowel syndrome, stroke, atherosclerosis, cancer and diseases where it is necessary to promote dendritic cell formation, hereinafter referred to as "the Diseases", amongst others. In another aspect the invention relates to Sema7A recombinant materials and methods for their production. In a further aspect, the invention relates to methods for identifying agonists and antagonists/inhibitors using the materials provided by the invention, and treating conditions associated with Sema7A imbalance with the identified compounds In a still further aspect, the invention relates to diagnostic assays for detecting diseases associated with inappropriate Sema7A activity or levels.

Description of the Invention

In a first aspect, the present invention relates to the use of a compound selected from:

(a) a Sema7A polypeptide;

(b) a compound which activates a Sema7A polypeptide;

(c) a compound which inhibits a Sema7A polypeptide; or

(d) a polynucleotide encoding a Sema7A polypeptide

for the manufacture of a medicament for treating:

(i) autoimmune disorders,

(ii) chronic obstructive pulmonary disease;

(ii) inflammation;

(iii) psoriasis;

(iv) tissue repair;

(v) wounds, to enhance wound healing;

(vi) irritable bowel syndrome;

(vii) stroke;

(viii) atherosclerosis;

(ix) cancer; or

(x) diseases where it is necessary to promote dendritic cell formation.

Such Sema7A polypeptides include isolated polypeptides comprising an amino acid sequence which has at least 95% identity, preferably at least 97-99% identity, to that of SEQ ID NO:2 over the entire length of SEQ ID NO:2. Such polypeptides include those comprising the amino acid of SEQ ID NO:2.

Further polypeptides of the present invention include isolated polypeptides in which the amino acid sequence has at least 95% identity, preferably at least 97-99% identity, to the amino acid sequence of SEQ ID NO:2 over the entire length of SEQ ID NO:2. Such polypeptides include the polypeptide of SEQ ID NO:2.

Further peptides of the present invention include isolated polypeptides encoded by a polynucleotide comprising the sequence contained in SEQ ID NO:1.

Polypeptides of the present invention are members of the semaphorin family of polypeptides. The semaphorin polypeptides were originally implicated in growth cone guidance in the embryonic nervous system (Culotti and Kolodkin, Curr. Opin. Neurobiol. 6:81-88, 1996). However, recent publications have shown that other semaphorin polypeptides have an immune function: M-Sema-G and CD100 (Furuyama et al. J Biol. Chem. 271:33376-81, 1996; Hall et al. PNAS 93:11780-85, 1996).

Sema7A is associated with the cell surface through a glycophosphatidylinositol linkage and is highly homologous to the semaphorin encoded by alcelaphine herpesvirus-1 (46%). Homology to all other known semaphorins is 30% or less. The viral semaphorins (vaccinia and alcelaphine herpesvirus) and Sema7A have recently been shown to bind to a plexin like receptor VESPR (Plexin-C1; Tamagnone et al. Cell 99, 71-80, 1999). VESPR is a 200 kDa transmembrane glycoprotein that may signal by associating a tyrosine kinase.

These properties are hereinafter referred to as "Sema7A activity" or "Sema7A polypeptide activity" or "biological activity of Sema7A". Also included amongst these activities are antigenic and immunogenic activities of said Sema7A polypeptides, in particular the antigenic and immunogenic activities of the polypeptide ofSEQ ID NO:2. Preferably, a polypeptide of the present invention exhibits at least one biological activity of Sema7A.

The polypeptides of the present invention may be in the form of the "mature" protein or may be a part of a larger protein such as a precursor or a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-

sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The present invention also includes variants of the aforementioned polypeptides, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acids are substituted, deleted, or added in any combination.

Polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

In a further aspect, the present invention relates to Sema7A polynucleotides. Such polynucleotides include isolated polynucleotides comprising a nucleotide sequence encoding a polypeptide which has at least 95% identity to the amino acid sequence of SEQ ID NO:2, over the entire length of SEQ ID NO:2. In this regard, polypeptides which have at least 97% identity are highly preferred, whilst those with at least 98-99% identity are more highly preferred, and those with at least 99% identity are most highly preferred. Such polynucleotides include a polynucleotide comprising the nucleotide sequence contained in SEQ ID NO:1 encoding the polypeptide of SEQ ID NO:2.

Further polynucleotides of the present invention include isolated polynucleotides comprising a nucleotide sequence that has at least 95% identity to a nucleotide sequence encoding a polypeptide of SEQ ID NO:2, over the entire coding region. In this regard, polynucleotides which have at least 97% identity are highly preferred, whilst those with at least 98-99% identity are more highly preferred, and those with at least 99% identity are most highly preferred.

Further polynucleotides of the present invention include isolated polynucleotides comprising a nucleotide sequence which has at least 95% identity to SEQ ID NO:1 over the entire length of SEQ ID NO:1. In this regard, polynucleotides which have at least 97% identity are highly preferred, whilst those with at least 98-99% identiy are more highly preferred, and those with at least 99% identity are most highly preferred. Such polynucleotides include a polynucleotide comprising the polynucleotide of SEQ ID NO:1 as well as the polynucleotide of SEQ ID NO:1.

The invention also provides polynucleotides which are complementary to all the above described polynucleotides.

The nucleotide sequence of SEQ ID NO:1 shows homology with alcelaphine herpesvirus type-1 (Ensser & Fleckstein, J Gen. Virology 1995, 76:1063-7 GenBank U18243). The nucleotide sequence of SEQ ID NO:1 is a cDNA sequence and comprises a polypeptide encoding sequence (nucleotide 13 to 2010) encoding a polypeptide of 666 amino acids, the polypeptide of SEQ ID NO:2. The nucleotide sequence encoding the polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in SEQ ID NO:1 or it may be a sequence other than the one contained in SEQ ID NO:1, which, as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2. The polypeptide of the SEQ ID NO:2 is structurally related to other proteins of the semaphorin family, having homology and/or structural similarity with alcelaphine herpesvirus type-1 (Ensser & Fleckstein, J Gen. Virology 1995, 76:1063-7). The gene encoding the Sema7A polypeptide of SEQ ID NO:2 has been localised to human chromosome 15q22.3-q23 (Lange et al Genomics 1998, 51:340-350).

Preferred polypeptides and polynucleotides of the present invention are expected to have, *inter alia*, similar biological functions/properties to their homologous polypeptides and polynucleotides. Furthermore, preferred polypeptides and polynucleotides of the present invention have at least one Sema7A activity.

Polynucleotides of the present invention may be obtained, using standard cloning and screening techniques, from a cDNA library derived from mRNA in cells of myeloid origin (Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

When polynucleotides of the present invention are used for the recombinant production of polypeptides of the present invention, the polynucleotide may include the coding sequence for the mature polypeptide, by itself; or the coding sequence for the mature polypeptide in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, Proc Natl Acad Sci USA (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further embodiments of the present invention include polynucleotides encoding polypeptide variants which comprise the amino acid sequence of SEQ ID NO:2 and in which several, for instance from 5 to 10, 1 to 5, 1 to 3, 1 to 2 or 1, amino acid residues are substituted, deleted or added, in any combination.

Recombinant polypeptides of the present invention may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to expression systems which comprise a polynucleotide or polynucleotides of the present invention, to host cells which are genetically engineered with such expression sytems and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al, Basic Methods in Molecular Biology (1986) and Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). Preferred such methods include, for instance, calcium phosphate transfection, DEAE-dextran mediated transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as *Streptococci*, *Staphylococci*, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used, for instance, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector which is able to maintain, propagate or express a polynucleotide to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, Molecular Cloning, A

Laboratory Manual (supra). Appropriate secretion signals may be incorporated into the desired polypeptide to allow secretion of the translated protein into the lumen of the endoplasmic reticulum, the periplasmic space or the extracellular environment. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If a polypeptide of the present invention is to be expressed for use in screening assays, it is generally preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If the polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide. If produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

This invention also relates to the use of polynucleotides of the present invention as diagnostic reagents. Detection of a mutated form of the gene characterised by the polynucleotide of SEQ ID NO:1 which is associated with a dysfunction will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, or susceptibility to a disease, which results from under-expression, over-expression or altered expression of the gene. Individuals carrying mutations in the gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeledSema7A nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing (ee, e.g., Myers *et al.*, Science (1985) 230:1242). Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (see Cotton *et al.*, Proc Natl Acad Sci

USA (1985) 85: 4397-4401). In another embodiment, an array of oligonucleotides probes comprising Sema7A nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see for example: M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility tothe Diseases through detection of mutation in the Sema7A gene by the methods described. In addition, such diseases may be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of polypeptide or mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, nucleic acid amplification, for instance PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as a polypeptide of the present invention, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Thus in another aspect, the present invention relates to a diagonostic kit which comprises:
(a) a polynucleotide of the present invention, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment thereof ;
(b) a nucleotide sequence complementary to that of (a);
(c) a polypeptide of the present invention, preferably the polypeptide of SEQ ID NO:2 or a fragment thereof; or
(d) an antibody to a polypeptide of the present invention, preferably to the polypeptide of SEQ ID NO:2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component. Such a kit will be of use in diagnosing a disease or suspectability to a disease, particularly chronic obstructive pulmonary disease, inflammation, psoriasis, wound healing, tissue repair, irritable bowel syndrome, stroke or atherosclerosis, amongst others.

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them, can also be used as immunogens to produce antibodies immunospecific for polypeptides of the present invention. The term " immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against polypeptides of the present invention may be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a non-human animal, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., Nature (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, Immunology Today (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, Monoclonal Antibodies and Cancer Therapy, 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies, such as those described in U.S. Patent No. 4,946,778, can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms, including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against polypeptides of the present invention may also be employed to treat the Diseases, amongst others.

In a further aspect, the present invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa. Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with a polypeptide of the present invention, adequate to produce antibody and/or T cell immune response to protect said animal from the Diseases hereinbefore mentioned, amongst others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering a polypeptide of the present invention *via* a vector directing expression of the

polynucleotide and coding for the polypeptide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

A further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a polypeptide of the present invention wherein the composition comprises a polypeptide or polynucleotide of the present invention The vaccine formulation may further comprise a suitable carrier. Since a polypeptide may be broken down in the stomach, it is preferably administered parenterally (for instance, subcutaneous, intramuscular, intravenous, or intradermal injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation instonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Polypeptides of the present invention are responsible for one or more biological functions, including one or more disease states, in particular the Diseases hereinbefore mentioned. It is therefore desirous to devise screening methods to identify compounds which stimulate or which inhibit the function of the polypeptide. Accordingly, in a further aspect, the present invention provides for a method of screening compounds to identify those which stimulate or which inhibit the function of the polypeptide. In general, agonists or antagonists may be employed for therapeutic and prophylactic purposes for such Diseases as hereinbefore mentioned. Compounds may be identified from a variety of sources, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. Such agonists, antagonists or inhibitors so-identified may be natural or modified substrates, ligands, receptors, enzymes, etc., as the case may be, of the polypeptide; or may be structural or functional mimetics thereof (see Coligan *et al.*, Current Protocols in Immunology 1(2):Chapter 5 (1991)).

The screening method may simply measure the binding of a candidate compound to the polypeptide, or to cells or membranes bearing the polypeptide, or a fusion protein thereof by means of a label directly or indirectly associated with the candidate compound. Alternatively, the screening method may involve competition with a labeled competitor. Further, these screening

methods may test whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide, using detection systems appropriate to the cells bearing the polypeptide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Constitutively active polpypeptides may be employed in screening methods for inverse agonists or inhibitors, in the absence of an agonist or inhibitor, by testing whether the candidate compound results in inhibition of activation of the polypeptide. Further, the screening methods may simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide of the present invention, to form a mixture, measuring Sema7A activity in the mixture, and comparing the Sema7A activity of the mixture to a standard. Fusion proteins, such as those made from Fc portion and Sema7A polypeptide, as hereinbefore described, can also be used for high-throughput screening assays to identify antagonists for the polypeptide of the present invention (see D. Bennett *et al.*, J Mol Recognition, 8:52-58 (1995); and K. Johanson *et al.*, J Biol Chem, 270(16):9459-9471 (1995)).

The polynucleotides, polypeptides and antibodies to the polypeptide of the present invention may also be used to configure screening methods for detecting the effect of added compounds on the production of mRNA and polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents which may inhibit or enhance the production of polypeptide (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The polypeptide may be used to identify membrane bound or soluble receptors, if any, through standard receptor binding techniques known in the art. These include, but are not limited to, ligand binding and crosslinking assays in which the polypeptide is labeled with a radioactive isotope (for instance, ^{125}I), chemically modified (for instance, biotinylated), or fused to a peptide sequence suitable for detection or purification, and incubated with a source of the putative receptor (cells, cell membranes, cell supernatants, tissue extracts, bodily fluids). Other methods include biophysical techniques such as surface plasmon resonance and spectroscopy. These screening methods may also be used to identify agonists and antagonists of the polypeptide which compete with the binding of the polypeptide to its receptors, if any. Standard methods for conducting such assays are well understood in the art.

The present invention provides evidence that Sema7A polypeptides may be active only when two Sema7A monomers are associated to form a homodimer or multimer. Thus a further screen comprises an assay wherein the ability of a compound to promote or inhibit

multimerisation is determined. Compounds that promote multimerisation are expected to have agonist activity, by increasing Sema7A activity *in-vivo* whereas compounds that inhibit multimerisation are expected to act as antagonists by decreasing Sema7A activity *in-vivo*. The format of assays involving dimerisation of two polypeptides are well known in the art.

Examples of potential polypeptide antagonists include neutralising antibodies, inactive monomeric Sema7A polypeptides or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, receptors, enzymes, etc., as the case may be, of the polypeptide, e.g., a fragment of the ligands, substrates, receptors, enzymes, etc.; or small molecules which bind to the polypetide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented. Antagonists or inhibitors of Sema7A, in the context of the present invention, are understood to include compounds which inactivate or inhibit the Sema7A monomeric or dimeric polypeptide directly and also compounds which inactivate or inhibit Sema7A indirectly by preventing dimerisation or multimerisation into an active form.

Examples of agonists include active dimeric or multimeric Sema7A complexes.

Thus, in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for polypeptides of the present invention; or compounds which decrease or enhance the production of such polypeptides, which comprises:

(a) a polypeptide of the present invention;

(b) a recombinant cell expressing a polypeptide of the present invention;

(c) a cell membrane expressing a polypeptide of the present invention; or

(d) antibody to a polypeptide of the present invention;

which polypeptide is preferably that of SEQ ID NO:2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

It will be readily appreciated by the skilled artisan that a polypeptide of the present invention may also be used in a method for the structure-based design of an agonist, antagonist or inhibitor of the polypeptide, by:

(a) determining in the first instance the three-dimensional structure of the polypeptide;

(b) deducing the three-dimensional structure for the likely reactive or binding site(s) of an agonist, antagonist or inhibitor;

(c) synthesing candidate compounds that are predicted to bind to or react with the deduced binding or reactive site; and

(d) testing whether the candidate compounds are indeed agonists, antagonists or inhibitors.

It will be further appreciated that this will normally be an interative process.

In a further aspect, the present invention provides methods of treating abnormal conditions such as, for instance, chronic obstructive pulmonary disease, inflammation, psoriasis, wound healing, tissue repair, irritable bowel syndrome, stroke or atherosclerosis,related to either an excess of, or an under-expression of, Sema7A polypeptide activity.

If the activity of the polypeptide is in excess, several approaches are available. One approach comprises administering to a subject in need thereof an inhibitor compound (antagonist) as herein above described, optionally in combination with a pharmaceutically acceptable carrier, in an amount effective to inhibit the function of the polypeptide, such as, for example, by blocking the binding of ligands, substrates, receptors, enzymes, etc., or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of the polypeptides still capable of binding the ligand, substrate, enzymes, receptors, etc. in competition with endogenous polypeptide may be administered. Typical examples of such competitors include fragments of the Sema7A polypeptide.

In still another approach, expression of the gene encoding endogenous Sema7A polypeptide can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or externally administered (see, for example, O'Connor, J Neurochem (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Alternatively, oligonucleotides which form triple helices ("triplexes") with the gene can be supplied (see, for example, Lee *et al.*, Nucleic Acids Res (1979) 6:3073; Cooney *et al.*, Science (1988) 241:456; Dervan *et al.*, Science (1991) 251:1360). These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*. Synthetic antisense or triplex oligonucleotides may comprise modified bases or modified backbones. Examples of the latter include methylphosphonate, phosphorothioate or peptide nucleic acid backbones. Such backbones are incorporated in the antisense or triplex oligonucleotide in order to provide protection from degradation by nucleases and are well known in the art. Antisense and triplex molecules synthesised with these or other modified backbones also form part of the present invention.

In addition, expression of the human Sema7A polypeptide may be prevented by using ribozymes specific to the human Sema7A mRNA sequence. Ribozymes are catalytically active RNAs that can be natural or synthetic (see for example Usman, N, et al., Curr. Opin. Struct. Biol (1996) 6(4), 527-33.) Synthetic ribozymes can be designed to specifically cleave human Sema7A mRNAs at selected positions thereby preventing translation of the human Sema7A mRNAs into functional polypeptide. Ribozymes may be synthesised with a natural ribose phosphate

backbone and natural bases, as normally found in RNA molecules. Alternatively the ribosymes may be synthesised with non-natural backbones to provide protection from ribonuclease degradation, for example, 2'-O-methyl RNA, and may contain modified bases.

For treating abnormal conditions related to an under-expression of Sema7A and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates a polypeptide of the present invention, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of Sema7A by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For an overview of gene therapy, see Chapter 20, Gene Therapy and other Molecular Genetic-based Therapeutic Approaches, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996). Another approach is to administer a therapeutic amount of a polypeptide of the present invention in combination with a suitable pharmaceutical carrier.

In a further aspect, the present invention provides for pharmaceutical compositions comprising a therapeutically effective amount of a polypeptide, such as the soluble form of a polypeptide of the present invention, agonist/antagonist peptide or small molecule compound, in combination with a pharmaceutically acceptable carrier or excipient. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The composition will be adapted to the route of administration, for instance by a systemic or an oral route. Preferred forms of systemic administration include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if a polypeptide or other compounds of the present invention can be formulated in an enteric or an

encapsulated formulation, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels, and the like.

The dosage range required depends on the choice of peptide or other compounds of the present invention, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 μg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Polynucleotide and polypeptide sequences form a valuable information resource with which to identify further sequences of similar homology. This is most easily facilitated by storing the sequence in a computer readable medium and then using the stored data to search a sequence database using well known searching tools, such as those in the GCG or Lasergene software packages. Accordingly, in a further aspect, the present invention provides for a computer readable medium having stored thereon a polynucleotide comprising the sequence of SEQ ID NO:1 and/or a polypeptide sequence encoded thereby.

The following definitions are provided to facilitate understanding of certain terms used frequently herein before.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term "polynucleotide" also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications may be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications may occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present to the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from post-translation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, biotinylation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation

of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination (see, for instance, Proteins - Structure and Molecular Properties, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993; Wold, F., Post-translational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in Post-translational Covalent Modification of Proteins, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", Meth Enzymol (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Post-translational Modifications and Aging", Ann NY Acad Sci (1992) 663:48-62).

"Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in*

Molecular Biology, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM *J. Applied Math., 48:* 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., *Nucleic Acids Research 12(1):* 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., *J. Molec. Biol. 215:* 403-410 (1990). The BLAST X program is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., *et al.*, NCBI NLM NIH Bethesda, MD 20894; Altschul, S., *et al., J. Mol. Biol. 215*: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.

Preferred parameters for polypeptide sequence comparison include the following:.

1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, Proc. Natl. Acad. Sci. USA. 89:10915-10919 (1992)

Gap Penalty: 12

Gap Length Penalty: 4

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

Preferred parameters for polynucleotide comparison include the following:

1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

Available as: The "gap" program from Genetics Computer Group, Madison WI. These are the default parameters for nucleic acid comparisons.

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:1, that is be 100% identical, or it may include up to a certain integer number of nucleotide alterations as compared to the reference sequence. Such alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal

positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the numerical percent of the respective percent identity(divided by 100) and subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \bullet y),$$

wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides in SEQ ID NO:1, and y is, for instance, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%,etc., and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n. Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

Similarly, a polypeptide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2, that is be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the % identity is less than 100%. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the numerical percent of the respective percent identity(divided by 100) and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \bullet y),$$

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ ID NO:2, and y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a.

"Homolog" is a generic term used in the art to indicate a polynucleotide or polypeptide sequence possessing a high degree of sequence relatedness to a subject sequence. Such relatedness may be quantified by determining the degree of identity and/or similarity between the

sequences being compared as hereinbefore described. Falling within this generic term are the terms "ortholog", meaning a polynucleotide or polypeptide that is the functional equivalent of a polynucleotide or polypeptide in another species, and "paralog" meaning a functionally similar sequence when considered within the same species.

"Fusion protein" refers to a protein encoded by two, often unrelated, fused genes or fragments thereof. In one example, EP-A-0 464 discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, employing an immunoglobulin Fc region as a part of a fusion protein is advantageous for use in therapy and diagnosis resulting in, for example, improved pharmacokinetic properties [see, e.g., EP-A 0232 262]. On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified.

Examples

Example 1 – cloning of Sema7A

The complete Sema7A coding sequence (GenBank accession number AF030698) was amplified by RT-PCR as follows. Oligo dT-primed first-strand cDNAs were made from 1μg human placental poly-A mRNA (Clontech), using Superscript II reverse transcriptase (Life Technologies) according to the manufacturer's instructions. Oligonucleotide primers designed to amplify the coding sequence of human Sema7A (forward 5'-ACC ATG ACG CCT CCT CCG C-3', reverse 5'-GCT CTG AGT GTG AGA CGT TC-3') were used for PCR from 1/500th of the RT reaction product. 1/25th of the PCR product was then further amplified in a PCR that used the same forward primer and a nested reverse primer (5'-CAG AAG CCT GAG GCA TGC-3'). Final concentrations of reagents in both PCRs were 0.2mM dNTP, 40units/ml of *PfuTurbo* DNA polymerase (Stratagene), 1x *PfuTurbo* PCR buffer and 12pmol PCR primers. The thermal cycle used for both PCR reactions began at 94°C for 2 min, followed by eight cycles of 94°C for 10 s, 63°C for 30 s, and 72°C for 2 min, twenty cycles of 94°C for 10 s, 58°C for 30 s, and 72°C for 3 min, and ended with a further 3 min at 72°C. PCR products were separated by agarose gel electrophoresis. Those of the size expected for Sema7A were purified by a silica-based method (Qiaquick Kit, Qiagen), cloned into the topoisomerase-activated pcDNA3.1-topo vector (Invitrogen), and transformed into *E. coli* K12 TOP10 (Invitrogen). Clones containing the plasmid with the Sema7A insert in the correct orientation were identified by PCR colony screening. Plasmid DNA was prepared from suspension cultures of selected clones by alkaline lysis followed by anion-exchange chromatography (Qiagen Miniprep Kit, Qiagen).
Clones were sequenced on both strands from standard vector primers and internal gene-specific primers, using an ABI automated sequencer. Sequences were assembled with a software package, Seqman (DNASTAR), and the alignments optimised manually to give an overall consensus.

Example 2 - Vector Construction.

The C terminal end of Sema7A contains a hydrophobic region which is cleaved off during the addition of the GPI anchor (Xu *et al.* (1998) J.Biol.Chem. 273, 22428-22434). The truncated Sema7A gene (missing 25AA from the C terminal end) was PCR amplified using the full-length clone from example 1 as the template. Primers were designed with unique restriction sites at both the N' and C' terminal ends of the gene to allow efficient sub-cloning into the expression vector. PCR amplification was carried out using Pfu Turbo DNA polymerase using cycling conditions as recommended by the manufacturer (Stratagene). The addition of 5% DMSO was required for efficient PCR due to the high % GC ratio of the gene. The PCR product was of expected size and

was cloned into the PCR cloning vector TOPO TA as per instructions of the manufacturer (Invitrogen). The expression vector, pIL4/FcLinkXa, was altered by replacing the factor Xa cleavage site with the PreScission cleavage site (Pharmacia). The IL4 leader sequence was also removed on sub-cloning as the Sema7A gene had its own signal sequence. The fidelity of the gene and vector construction was confirmed by DNA sequencing on both strands using an ABI 377 Automative DNA Sequencer (Applied Biosystems).

Example 3 - Generation Stable mass cultures in CHOE1a.

Large-scale plasmid DNA isolation of the clone pFcLinkPrec/Sema7A and the empty vector control pFcLinkPrec was carried out using maxi-preps (Qiagen) as per instructions of the manufacturer. Concentration of the plasmid DNA was determined by OD 260/280nm and the fidelity of the clones determined by restriction endonuclease digestion. Transfection of pFcLinkPrec/Sema7A and also the control vector pFcLinkPrec into CHOE1A cells was performed using electroporation ($3x10^7$cells, 0.4cm gap cuvette, 380V-25μF). Cell lines were maintained after transfection in MR1-4 medium + 1 X nucleosides. Selection was commenced 48h post-transfection in MR1-4 medium (no nucleosides). Media was changed every 48 to 72h until stable mass cultures were obtained (4 weeks). The supernatant of the stable mass of both pFcLinkPrec/Sema7A and the control pFcLinkPrec were evaluated for secreted protein expression after 72h growth.

Example 4 - Culture and purification of Sema7A-Fc.

The CHO_Sema7A-Fc cells were routinely maintained in shake flask cultures which were shaken at 100-120 rpm, incubated at 37°C in an atmosphere of 5% CO_2 in air. The cells were grown in a proprietary serum free medium and were maintained at a cell concentration of between 2-$30x10^6$ cells/ml. For production of recombinant protein, the cultures were scaled up into 1L shake flasks and grown to a cell concentration of approximately $8x10^5$ cells/ml, at which point the cultures were transferred to 34°C for the duration of the production. The cultures were harvested for product by centrifugation (2000g, 15min) before the cell viability dropped below 85%. Routinely the cell viability at harvest was in excess of 90%. The supernatant containing the recombinant protein was stored sterile at 4°C prior to purification. Purification was accomplished by capture of conditioned media on protein A (ProsepA; BioProcessing, UK), washing with PBS followed by elution with 25mM Citrate pH 3.5. Eluted fractions were immediately neutralised with 500mM Tris pH 9.0 (by titration to pH 7.5). Yield of Sema7A-Fc was in the range of 20mg-40mg per litre of conditioned media. Cleavage of the Fc was accomplished by buffer exchange

into 50mM Tris pH 7.0, 150mM NaCl , 1mM EDTA ,1mM DTT and addition of 16 EU PreScission protease per 25mg of Sema7A-Fc and incubation for 16h at 4°C. The cleaved material was then passed over a second ProsepA column and the non adsorbed material collected and concentrated by stirred cell UF.

Example 5 – localisation of Sema7A

Using Taqman analysis, Sema7A mRNA was localised to a small extent in brain, lymphocytes and bone marrow and to a much larger extent in lung, placenta and macrophages using a Taqman masterplate (Poly A+ RNA from multiple tissues of four different individuals was isolated and the cDNA prepared. TaqMan PCR was performed to detect either Sema7A or housekeeping genes (Sarau et al. Mol. Pharmacol. 56, 657-663, 1999). A total of 21 tissues were tested: Enriched Brain (2 males, 2 females),Whole Brain (2 males only), Hypothalamus (2 males, 2 females), Spinal cord (2 males, 2 females), Heart (2 males, 2 females), Lung,(2 males, 2 females), Liver (2 males, 2 females), Foetal Liver (2 males, 2 females), Kidney (2 males, 2 females), Skeletal muscle,(2 males, 2 females), Small intestine (1 male, 2 females), Colon (2 males, 1 female), Spleen (2 males, 2 females), Peripheral Blood Lymphocytes (2 males, 2 females), Macrophages (2 males, 2 females), Adipose (1 male, 2 females), Pancreas (2 males, 2 females), Prostate/Placenta (2 males/2 females), Cartilage,(1 male, 2 females), Bone marrow (1 male, 1 female). Apart from the aforementioned Sema7A expression in brain, lymphocytes, bone marrow, lung, placenta and macrophages, Sema7A was not detected in the other tissues.

Example 6 – Preparation of monocytes, B cells, T cells and neutrophils

Human monocytes were prepared from healthy donors by an indirect or direct purification scheme using kits purchased from Miltenyi Biotec (similar results being obtained from both procedures). The monocytes were >90% pure by flow cytometry analysis. The cells (400K/well) were cultured in 96-well plates in RPMI 1640 with 10% heat inactivated autologous serum. The media was harvested as indicated for IL-1β, IL-6, IL-8 or TNFα analysis. To examine M-CSF production monocytes were cultured in wells for 5d that had been coated with or without Sema7A-Fc (2ug/ml) for 1h at 37C then blocked with 10% serum in RPMI 1640. Human neutrophils were prepared by the

method of Merritt *et al.* (1998) J.Biol.Chem., **264**, 1522-1527. B and T cell preparations were purified using kits supplied by Miltenyi Biotec.

Example 7 – Taqman analysis of monocyte/ macrophage cells and neutrophils

Taqman analysis of monocyte/macrophage cells revealed that Sema7A is present in monocytes and expression remains unchanged after 1 day in culture with 2% human serum. However, after 4 days expression increases to 4-fold over naive levels, suggesting an increased role in mature macrophages.

Taqman analysis of neutrophils revealed that Sema7A was detected at low levels in freshly isolated neutrophils. Expression was tripled upon attachment of the cells to plastic, and was further increased with 20 nM GM-CSF treatment, up to 10-fold at 1 hour.

Example 8 – Detection of Sema7A protein

Sema7A protein was identified on B cells by flow cytometry but not detected on monocytes or other PBL's. Sema7A is expressed in monocytes and neutrophils and probably released through proteolysis and acts as a very potent autocrine activator of monocytes and neutrophils.

Example 9 - Monocyte superoxide release assay and chemotaxis.

To each well was added 50ul of monocytes ($5x10^6$/ml) in DPBS containing 0.5mM $CaCl_2$ and 0.2% BSA, 25ul of 0.6mM cytochrome C and 25ul of test compound. The plate was then read at 550nm, 37°C. Absorbance at 550nm was converted to nanomoles O_2^-, based on the extinction coefficient of (reduced minus oxidised) cytochrome C, $E_{550nm} = 21 \times 10^3 M^{-1}cm^{-1}$.

Sema7A stimulates superoxide release at pM concentrations, and is similar in potency to fMLP. Sema7A has distinct differences to MCP-1 which has no effect on superoxide release and stimulates Ca transients (EC_{50} 1.5 ± 0.2nM) whereas Sema7A had no effect on Ca transients in monocytes (data not shown).

Cell migration was evaluated using a 48-well modified Boyden microchemotaxis chamber as described in Berkhout et al (1997) J. Biol. Chem., **272**, 16404-16413. Sema7A induces monocyte chemotaxis at fM concentrations and has an EC_{50} of 0.4pM which is 1000 times more potent than MCP-1. EC50 value for MCP-1-stimulated human

monocyte chemotaxis is 0.4 ± 0.01nM. Sema7A was less effective in stimulating neutrophil chemotaxis, EC_{50} 20pM.

Example 10 – Stimulation of cytokine production by Sema7A

Cytokine immunoassays.

96 well plates were coated with 1ug/ml of anti-cytokine monoclonal antibody in PBS overnight at 4°C. Following blocking and washing the wells were incubated for 60min shaking at RT with 50ul standard/unknown plus 50ul 0.5ug/ml biotin anti-cytokine monoclonal antibody in assay buffer. After washing, the wells were incubated for 30min shaking at RT with Eu^{3+} labelled streptavidin (Wallac), wells washed and enhancer (Wallac) added and the time-resolved fluorescence measured. Anti-cytokine paired monoclonal antibodies were purchased from Pharmingen or prepared in-house (IL-4, IL-5 and IL-18).

Sema7A at 10pM significantly stimulated monocyte inflammatory cytokine production (IL-1β, IL-6, IL-8 and TNFα) but had no effect on IL-10, IL-12 or IL-18 stimulation (up to 10nM) from monocytes; this effect was not due to LPS as heating Sema7A destroyed all activity. Maximal stimulation was observed with approximately 10nM Sema7A which was equivalent to stimulation with a maximal concentration of LPS. In similar experiments it was shown that Sema7A had no effect on cytokine release from T cells (IFN gamma, IL2, IL4, IL5) with or without T cell activators and Sema7A also has no effect on B cell cytokine release (IL6, TNFα, TNFβ, IL10).

Dependent on the donor cell preparation Sema7A is active at 1-10pM in stimulating proinflammatory cytokines from monocytes (Table 1). In all experiments controls of heat inactivated Sema7A/Sema7A-Fc (to test for the presence of low amounts of endotoxin) were included and no protein preparations gave any stimulation of cytokine production. Sema7A and Sema7A-Fc are equally effective at stimulating proinflammatory cytokines from monocytes, maximum activity being approximately 10nM. Sema7A has no effect on IL-10, IL-12 or IL-18 secretion from monocytes/macrophages (data not shown). Monocytes cultured for 4 days are far less responsive to Sema7A then the 1 day culture (Table 1). Sema7A was also less effective (approximately 20-fold) at stimulating proinflammatory cytokines from neutrophils compared to monocytes (Table 2). Sema7A-Fc in solution has a much greater effect on stimulating monocyte cytokine release (GM-

CSF, IL-6, IL-8, IL-1β and TNFα) than immobilised Sema-7A-Fc (Table 3). Sema7A-Fc immobilised on tissue culture dishes, stimulated the production of M-CSF to a far greater extent than solution phase Sema7A-Fc. Levels after 5 days of culture exceeded 14.5ng/ml. Monocytes exposed to immobilised CD80-Fc, used as a control for Fc receptor mediated effects, produced the same level of M-CSF as untreated cells (0.4 ng/ml). These results indicate that Sema7A stimulation of M-CSF production in monocytes probably requires cross-linking with its receptor.

Table 1. Effect of Sema7A on cytokine production from monocytes in culture for 1 or 4 days. Day 1 monocytes were exposed to Sema7A for 24h whereas day 4 monocytes were cultured in media only for 3 days then exposed to Sema7A for 24h. Results are the mean of triplicate determinations, SD < 5%.

	Cytokine, ng/ml			
condition	IL-6	IL1β	IL-8	TNFα
day 1 monocytes;	0	0.1	28	0
day 1 monocytes; + Sema7A, 1pM	0	0.1	32	0.1
day 1 monocytes; + Sema7A, 10pM	1.3	0.2	41	0.5
day 1 monocytes; + Sema7A, 100pM	17.5	1.3	116	6
day 1 monocytes; + Sema7A, 1nm	236	22.6	903	25
day 4 monocytes;	0	0	0	
day 4 monocytes; + Sema7A, 1pM	0	0	3	
day 4 monocytes; + Sema7A, 10pM	0	0	13	
day 4 monocytes; + Sema7A, 100pM	6	0	36	
day 4 monocytes; + Sema7A, 1nm	14	0.1	74	

Table 2. Effect of Sema7A-Fc on proinflammatory cytokine production from neutrophils. Results are the mean of triplicate determinations, SD<5%.

		Cytokine, ng/ml			
Cells	Treatment	IL-1β	IL-6	IL-8	TNFα

neutrophils	control	0	0	0.2	0
	Sema7A-Fc, 1nM	0	0.1	0.5	0
	Sema7A-Fc,10nM	0.3	11.2	15.1	0.4

Table 3. Effect of Sema7A-Fc on cytokine production from 5 day monocyte cultures. Results are the mean of triplicate determinations, SD < 5%. Sema7A-Fc (10nM) was added in solution or plate bound for 5 days and the cytokine profile assessed by immunoassay.

	Cytokine, pg/ml					
monocytes (day 5)	GM-CSF	M-CSF	IL-6	IL-8	TNFα	IL-1β
control	3	350	4	0	0	0
CD80-Fc	2	402	3	0	0	0
Sema7A-Fc (plate)	155	14570	5514	3589	694	246
Sema7A-FC (solution)	397	1032	8018	5989	2290	492

Example 11 - Flow cytometry analysis of Sema7A stimulated monocytes

After 5 days in culture in the presence of immobilised Sema7A-Fc, the monocytes show a trend to becoming CD11b+/CD14-/CD1a-/CD83+/CD40+/CD86+ dendritic cells. Sema7A also increases monocyte cell survival, due to stimulation of M-CSF and G-MCSF.
The Sema7A-Fc data was compared to immobilised CD80-Fc as a control for FC mediated effects. Similar results were obtained from cells of 3 different donors .

Example 12 – Effect of Sema7A *in-vivo*

Sema7A has dramatic *in vivo* effects in the mouse. Sema7A given i.p. significantly increased the recruitment of neutrophils into the peritoneum of the mouse after 4h ($7.1 \pm 3.2 \times 10^5$ cells vs $0.9 \pm 0.5 \times 10^5$ cells in the vehicle control) and at 24h ($12.3 \pm 3 \times 10^5$ cells vs $0.1 \pm 0.05 \times 10^5$ cells in the vehicle control) but had no significant effect on total leukocyte, lymphocytes, eosinophils, mast cells or monocyte numbers (Table 4). (In these experiments mice [6 per group] were injected i.p. with 1ml sterile PBS or 20ug Sema7A in 1ml PBS; PBS/EDTA was used to lavage the peritoneal cavity of killed mice at 4 or

24h post injection. Total leukocyte number was determined using a haemocytometer and differential cell counts performed on a Diff Quick stained cytospin preparation). Flow cytometry staining with anti-mouse F4/80 Mab (Ajuebor et al. J Leukocyte Biol. 1998, 63:108-115) on cells recovered 24h post injection revealed that in the controls only a single cell population was stained indicating the presence of resident macrophages only as expected. There was evidence of double staining in the Sema7A 24h sample group indicating that a proportion of the peritoneal monocytes/macrophages were recruited from the blood.

Table 4

Treatment	leukocytes cells x10^6	neutrophils cells x10^5	monocytes cells x10^6
control (4h)	3.7 ± 0.8	0.9 ± 0.5	3.2 ± 0.7
Sema7A (4h)	3.0 ± 1.3	**7.1 ± 3.2**	2.0 ± 0.8
control (24h)	1.3 ± 0.5	0.1 ± 0.05	1.2 ± 0.5
Sema7A (24h)	3.2 ± 0.5	**12 ± 3**	1.6 ± 0.3

values are Mean±SEM

Example 13 – Sema7A as an active homodimer

A purified Sema7A preparation from example 4 (from the high expressing CHOE1A cell line) was analysed by size exclusion chromatography and it was observed that the protein separated into two peaks. Both peaks were tested for bioactivity and it was found that only the high molecular weight Sema7A peak was biologically active. This result suggests that the Sema7A may be active only in the dimeric or multimeric form.

SEQUENCE INFORMATION

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Claims

1. The use of a compound selected from:

(a) a Sema7A polypeptide;

(b) a compound which activates a Sema7A polypeptide;

(c) a compound which inhibits a Sema7A polypeptide; or

(d) a polynucleotide encoding a Sema7A polypeptide,

for the manufacture of a medicament for treating:

(i) autoimmune disorders,

(ii) chronic obstructive pulmonary disease;

(ii) inflammation;

(iii) psoriasis;

(iv) tissue repair;

(v) wounds, to enhance wound healing;

(vi) irritable bowel syndrome;

(vii) stroke;

(viii) atherosclerosis;

(ix) cancer; or

(x) diseases where it is necessary to promote dendritic cell formation.

2. The use according to claim 1 wherein the medicament is used to treat chronic obstructive pulmonary disease.

3. The use according to claim 1 wherein the medicament is used to treat inflammation.

4. The use according to claim 1 wherein the medicament comprises an isolated polypeptide which comprises a polypeptide having at least 95% identity to the Sema7Apolypeptide of SEQ ID NO:2.

5. The use according to claim 4 wherein the isolated polypeptide is the Sema7Apolypeptide of SEQ ID NO:2.

6. The use according to claim 1 wherein the medicament comprises a compound which activates a Sema7A polypeptide.

7. The use according to claim 1 wherein the medicament comprises a polynucleotide encoding a polypeptide having at least 95% identity with the amino acid sequence of SEQ ID NO:2.

8. The use according to claim 7 wherein the polynucleotide comprises a polynucleotide having at least 95% identity with the polynucleotide of SEQ ID NO:1.

9. The use according to claim 7 or 8 wherein the polynucleotide has the polynucleotide sequence of SEQ ID NO:1.

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